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# Stem Cells: A Plant Biology Perspective

Meeting Report

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**A recent meeting at the Juan March Foundation in Madrid, Spain brought together plant biologists to discuss the characteristics of plant stem cells that are unique and those that are shared by stem cells from the animal kingdom.**

## Introduction

Stem cells have two key characteristics: the ability to form many differentiated cell types and the ability to self-renew such that one daughter cell remains a stem cell. Elucidating how populations of stem cells renew themselves while sustaining the formation of new tissues is crucial if we are to understand the development of multicellular plants and animals. The concept that cells surrounding stem cells create a unique microenvironment that acts as a 'niche' to maintain and nurture the stem cells was formulated long ago (Schofield, 1978). However it is only recently that studies on tractable stem cell populations in vivo, such as the *Drosophila* germline, have confirmed the existence of these niches and their importance for stem cell maintenance.

The study of plant stem cells has had a somewhat chequered history. It has long been known that plants contain "initial" cells in zones of cell division within their growing tips. Yet, curiously, these initial cells often were not considered special because of the exaggerated claim found in many textbooks that *all* plant cells are totipotent. Plant developmental biologists long ago accepted that plants have stem cells whose differentiating daughter cells can revert back to a stem cell fate under certain situations. However, it is only recently that the notion of stem cell reversal has been recognized for animal stem cell populations (Kai and Spradling, 2004). The discovery of "organizing" cells in close proximity to plant stem cells (van den Berg et al., 1997) has led to the realization that stem cell niches are found in both plants and animals, even though the multicellular states of plants and animals evolved independently. The characteristics that make plant stem cells unique as well as the similarities between plant and animal stem cells provided an exciting focus for the recent Juan March Foundation meeting.

## Plant Stem Cells

Plant stem cells were introduced to meeting participants by Ben Scheres (Utrecht University) with a description of those in the root tip of the model plant *Arabidopsis* (see Figure 1). Regular cell division patterns allow the unequivocal identification of all stem cells that produce the tissues of the plant's root. Quiescent cells in the center of the root's stem cell population are required to maintain the stem cell state (see Figure 1), reminiscent of the stem cell niches found in animal systems. The SHORTROOT and SCARECROW plant-specific transcription factors specify the position of quiescent cells along the radial axis. In contrast, two PLETHORA proteins, containing plant-specific DNA binding domains, specify the quiescent cell and stem cell region along the proximo-distal axis of the root tip (Aida et al., 2004). Given that this set of factors for stem cell patterning is plant specific, are there more general players that influence all stem cell populations? New data suggest that stem cell maintenance is exquisitely sensitive to the activity of the retinoblastoma protein and its signaling pathway, which act downstream of cues that specify quiescent cell fate. The retinoblastoma pathway has been implicated in mammalian stem cell proliferation (Liu et al., 2004), suggesting that divergent patterning mechanisms for somatic stem cells in plants and animals may be connected to similar stem cell maintenance factors.

Thomas Laux (Freiburg University) discussed stem cells in the plant shoot apical meristem that give rise to leaves, stems, and flowers. Laux pointed out that shoot stem cells are a subpopulation of a larger set of undifferentiated cells within the meristem. Stem cells are maintained by an underlying organizing center that expresses the *WUSCHEL (WUS)* gene, which encodes a transcription factor required for organizer function (Mayer et al., 1998) (see Figure 1). WUS controls transcription of a gene encoding the small protein CLAVATA3 (CLV3) that is only expressed in the stem cells that overlie the organizer; CLV3 represses the expression of WUS (Schoof et al., 2000). Laux reported that WUS needs additional inputs to activate CLV3 transcription during embryonic initiation of the stem cell niche. To search for common regulatory mechanisms between the shoot and root meristem niches, Laux and his collaborators analyzed the function of WOX5, a WUS homolog expressed in quiescent cells of the root. Subtle defects observed in *wox5* mutants indicate a role in quiescent cell specification and stem cell maintenance that is analogous to the role of *WUS* in the shoot. In addition, transcription of the *WOX5* gene is dependent on root patterning genes. These findings reveal similarities between the stem cell organizers of the root and shoot.

## Dynamic Interactions between Stem Cells and Their Organizers

In animals, at least a subset of well-characterized stem cell niches contain fixed organizer cells. In contrast, plant stem cell systems appear to be more dynamic. Rüdiger Simon (Düsseldorf University) elaborated on the regulatory loop between organizer cells expressing WUS and stem cells expressing CLV3 that controls stem cell number in the shoot apex (Brand et al., 2000; Schoof et al., 2000). The prevailing view is that orga-

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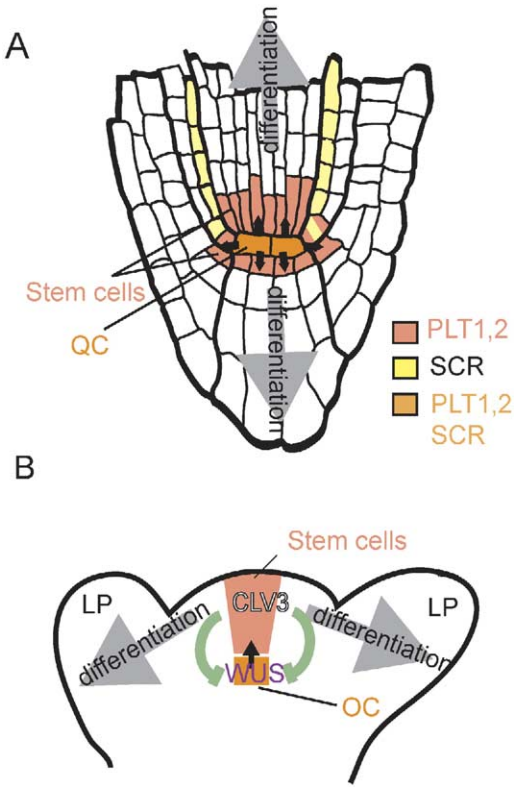


Figure 1. Plant Stem Cells and Their Niches

(A) In the *Arabidopsis* root, stem cells for all cell types surround the quiescent center (QC), a small group of organizer cells that are required for stem cell maintenance. Stem cells undergo asymmetric cell divisions and their daughter cells differentiate into all root cell types. Root stem cells and quiescent cells are specified by a combination of the PLETHORA (PLT1 and PLT2), SHORTROOT (SHR), and SCARECROW (SCR) transcription factors. Black arrows define a stem cell maintenance signal produced by quiescent cells that has yet to be identified.

(B) In the shoot of *Arabidopsis*, a stem cell pool is located above the organizer cells (OC), which express the WUSCHEL (WUS) transcription factor. The black arrow indicates an unknown signal that induces the organizer cells to express WUS. Stem cells of the plant shoot produce the small secreted protein CLAVATA3 (CLV3) that negatively regulates expression of WUS (green arrows). Stem cell daughters that leave the signaling domain differentiate and form leaf primordia (LP).

nizer cells that express WUS specify overlying stem cells expressing CLV3 through signals that have yet to be identified. CLV3 in turn represses WUS transcription by interacting with the more broadly expressed CLV1 leucine-rich-repeat receptor kinase signaling complex. This feedback loop could maintain stem cell number through an oscillatory mechanism. To test whether a dosage-sensitive feedback loop exists in the shoot apex, Simon's group used ethanol-inducible expression of the *CLV3* gene from its own promoter. They found that WUS expression as well as stem cell number appeared resistant to smaller changes in CLV3 production. These data suggest that the WUS-CLV3 feedback loop is not a simple oscillator but rather is dampened by as yet unknown factors that help to determine stem cell number.

Meanwhile, Fred Sack (Ohio State University) discussed involvement of the leucine-rich-repeat receptor-like protein TOO MANY MOUTH (TMM) in a stem cell-like compartment in developing leaves. In the leaf epidermis, some of these cells undergo repeated asymmetric divisions to establish specialized pairs of cells for gas exchange ("stomates"). The asymmetrically dividing cells are stem cells in the sense that they both self-renew and give rise to two different cell types (Nadeau and Sack, 2002). Mutation of the *TMM* gene reduces the number of divisions for self-renewal and disrupts oriented asymmetric division. TMM is expressed in leaf cells capable of division, and a pathway for regulating this division is now emerging. Newly identified components of this pathway include a potential ligand-cleaving subtilisin (von Groll et al., 2002) and the MAP kinase YODA (Bergmann et al., 2004). As the position of stomata is coupled to the plane of division of leaf cells that express TMM, the current model presumes that a TMM ligand is produced and processed in "organizing" stomatal precursor cells. This ligand, after interaction with a TMM-containing receptor complex that regulates MAPK signaling, instructs cell divisions in neighboring division-competent leaf cells.

## Setting Stem Cells apart

In plants, the shoot and root stem cell populations specified during embryogenesis give rise to organs that set aside new stem cell populations in an ordered pattern. Mature plant architecture largely depends on the spatial patterns of these lateral stem cell populations and the regulation of their activity.

The first plant stem cell precursors are specified during embryogenesis, and Gerd Jürgens (Tübingen University) presented data on the involvement of the plant hormone auxin in specifying the precursors for the organizing quiescent cells in the plant embryo. In *Arabidopsis*, the zygote divides into one cell that forms the embryo proper and a second cell that contributes to the extra-embryonic tissues; the uppermost derivative of this second cell gives rise to the quiescent cells. This recruitment requires the auxin response factor MONOPTEROS and is counteracted by the auxin-inducible repressor protein BODENLOS (Hamann et al., 2002). Jürgens provided evidence that auxin promotes BDL degradation via its receptor, the F box protein TIR1 (Dharmasiri et al., 2005). These data define an auxin signaling pathway in the embryonic lineage that instructs the extra-embryonic cell lineage to form the quiescent cell pool. Auxin transport to the extra-embryonic tissues by PIN proteins may be part of the signal. This fits with a role for *PLT* genes in quiescent cell specification as the expression of these genes is controlled by PIN-dependent auxin accumulation (Blilou et al., 2005).

In shoots, leaf primordia contain a lateral stem cell population. As the position of the lateral stem cell population in shoots depends on the positioning of organ primordia, regulation of leaf position ("phyllotaxis") appears to be the basic mechanism by which stem cells are set aside. Three presentations at the meeting dealt with this important issue.

Jan Traas (INRA Versailles) summarized evidence that auxin accumulation in leaf primordia determines

spacing of new primordia in the shoot apex. The Traas laboratory determined the positions of PIN proteins in the shoot apex and then imported these data into a computer program that calculated expected auxin distributions. The simulations predicted new auxin maxima at positions where new organ primordia were expected to arise. Traas' group is now building more elaborate models that incorporate cell division and growth characteristics to discover whether the position of new leaves can be programmed ab initio.

A refreshing change in the *Arabidopsis*-dominated plant sessions was wrought by David Jackson (Cold Spring Harbor Laboratory). His group has addressed the regulation of leaf position using a maize mutant called *aberrant phyllotyxy 1 (abph1)*. *ABPH1* encodes an *ARR* response regulator homolog that can be induced by cytokinins and is expressed in the apical meristem of the shoot (Giulini et al., 2004). Cytokinins are plant growth factors (not to be confused with cytokines) that have long been implicated in shoot apical meristem function, and ARRs are part of a cytokinin signal transduction pathway. One interpretation of the current data is that cytokinins promote expansion of the central zone of the shoot including expansion of stem cells and that ARRs limit this growth, thereby controlling the space available for initiation of leaf primordia.

Pilar Cubas (Madrid Centro Nacional of Biotechnology/CSIC) described two members of the TCP transcription factor family in *Arabidopsis*, BRC1 and BRC2, that delay lateral shoot development (Cubas et al., 1999). The BRC1 promoter is active in dormant buds, suggesting that repression of development and outgrowth is regulated in a cell-autonomous fashion by the *BRC1* gene. Cubas postulates that the *BRC* genes are local switches for growth that integrate environmental inputs to determine the pattern of lateral shoot activation that determines the final architecture of the plant. Detlef Weigel (MPI Tübingen) described various sets of microarray data that he has used to address functions of the TCP transcription factor family. His group has deduced potential targets by analyzing promoter motifs in genes that are regulated by the TCP family. Plant "multi"mutants carrying mutations in several *tcp* genes displayed defective transcription of many cell-cycle genes and aberrant exit from the cell cycle.

New data are emerging on the transmembrane receptor kinase AtSERK1 as revealed by Sacco de Vries (Wageningen University) at the meeting. This brassinosteroid co-receptor has been implicated in plant tissue regeneration in culture and potentially may be involved in reprogramming of stem cells. Expression data and functional analysis suggest that AtSERK1 may be involved in formation of procambial cells of the vasculature, in line with recently described roles for brassinosteroid receptors in vascular development (Cano-Delgado et al., 2004). But how is AtSERK1 involved in plant tissue regeneration? Vascular cells are known to have a remarkable potential for regeneration and are known to give rise to the cambial stem cells needed for secondary vascular growth. It may turn out that brassinolides, like auxins and cytokinins, may be involved in positioning plant stem cells in new locations, or perhaps the brassinosteroid pathway simply marks cells that are regeneration competent.

## Genomics for Stem Cell Research

It is debatable whether stem cells have unique transcriptional profiles. However, gene expression data from different types of stem cells as they begin to differentiate are certainly providing valuable insights into the undifferentiated state. Philip Benfey (Duke University) presented refined transcriptional profiling data from the *Arabidopsis* root that he sorted according to cell type and development zone (Birnbaum et al., 2003). Currently, this "digital in situ" encompasses most individual cell types, and refinements in the separation of quiescent cell and stem cell mRNA profiles are underway. High-throughput validation experiments using fusion of promoters and genes to green fluorescent proteins show that the data are reliable. The ultimate aim is to decipher transcriptional networks that define cell types and control cellular differentiation. Toward this end, Benfey's group have focused on the transcriptional network regulated by SHORTROOT (SHR), a transcription factor involved in the specification of quiescent cell identity. An inducible version of SHR, *shr* mutants, and ectopic SHR expression identified three sets of potential targets. Statistical meta-analysis (a method that combines confidence levels obtained from different experiments) identified eight targets with high confidence of which one, the SCARECROW (SCR) transcription factor, is already known to act downstream of SHR. Chromatin immunoprecipitation experiments validated these targets, demonstrating that this new combinatorial use of genomics tools is effective in deciphering transcriptional networks.

Robert Sablowski (John Innes Centre, Norwich, United Kingdom) and his group are investigating aspects of "stemness" that are shared by animals and plants. They have used gene profiling to identify new genes enriched in the stem cells of shoot apical meristems and in stem cell populations of the mouse. Using PSI-BLAST analysis, certain genes emerged whose expression is shared by both plant and animal stem cells. Annotation of these genes revealed that they encode cell-cycle regulators, DNA-repair proteins, and chromatin-remodeling factors. One of the more intriguing of these shared genes encodes an essential protein with a functional thioredoxin domain expressed in inflorescence and root meristems. Sablowski's group is now investigating whether this protein, which might protect against oxidative stress, is an essential feature of stem cells.

## Control of the Cell Cycle and Cell Differentiation

Meeting presentations revealed the complexity of plant gene families involved in cell-cycle control: for example, there are 10 A, 11 B, and 10 D cyclins predicted from the *Arabidopsis* genome. This complexity is beginning to be addressed systematically, which should facilitate analysis of cell-cycle control in plant stem cells and its involvement in cellular differentiation.

Dirk Inzé (VIB-University Ghent) and his team have used transcriptional profiling to unravel the order of gene activation after synchronized induction of lateral

root formation. Lateral root founder cells were sorted and profiled after synchronous induction. Auxin response factors (ARFs) and their inhibitory AUX/IAA partners, as well as several polar auxin transporters, were rapidly upregulated in the founder cells. In addition, the investigators noted upregulation of *cyclin A2*, *cyclin B1*, and *cyclin D3* genes. Crossreferencing these data with those from synchronized cells in suspension culture revealed that 60% of the genes were upregulated at the G2 to M transition of the cell cycle. Among these were genes that may be involved in cytoskeletal organization and cytokinesis. Inzé's group is now investigating 33 candidate genes for their possible involvement in the initial establishment of stem cell populations in the lateral root.

Crisanto Gutiérrez (Centro de Biologia Molecular Madrid) elaborated on downstream components involved in regulation of the transition from G1 to S in leaf cells. His group manipulated the activity of the RBR retinoblastoma homolog in plants, its downstream effectors E2Fa and E2Fc, as well as E2F targets, such as CDC6 and CDT1 (Castellano Mdel et al., 2004). In leaves, upregulation of proteins associated with the G1/S transition prolonged cell proliferation in division-competent cells. In differentiating cells, DNA endoreduplication—which leads to polyploid nuclei in mature cells—was stimulated. The genetic and biochemical tools generated in Gutiérrez 's lab can now be adopted to study regulation of the G1/S transition in stem cells. Arp Schnittger (University of Cologne) addressed the association of DNA endoreduplication with cell-fate determination and terminal cell differentiation. These investigators blocked DNA endoreduplication in polyploid leaf hairs (trichomes) by driving expression of the *CYCLIN D1* and *D3* genes from a late trichome promoter, resulting in the formation of multicellular yet correctly specified trichomes. However, when the *CYCLIN D1* and *D3* genes were driven from an early promoter, no trichomes were specified. Thus, at critical time points during development, the cell cycle is able to influence cell specification. When the KIP-RELATED PROTEIN 1 (KRP1) was misexpressed in trichomes, this protein moved to trichome-neighboring cells and induced premature DNA endoreduplication without interfering with their acquisition of a trichome cell fate. Highly endoreduplicated cells surrounding trichomes that misexpress KRP1 could re-enter the cell cycle and produce stomata (Weinl et al., 2005). Hence, endoreduplication is not irreversibly linked to terminal differentiation.

Jim Murray (University of Cambridge) reported on the homeobox gene *STM* that delays the differentiation of stem cells in the shoot apical meristem. Inducible overexpression of *STM* prolongs cell proliferation but cannot push differentiated cells back into the cell cycle; inducible silencing of *STM* leads to premature cell differentiation. Thus, STM may be a competence factor for the WUS-CLV3 stem cell maintenance loop, but direct connection to the cell cycle is so far lacking. Rob Martienssen (Cold Spring Harbor Laboratory) elaborated on STM and the antagonistic nuclear factors *AS1* and *AS2* that operate in emerging leaf primordia where cells embark on differentiation pathways. He showed that AS1 and AS2 not only antagonize STM and its close homologs in leaf primordia but also cooperate with these genes in the boundary region to regulate the expression of boundary-specific genes. This work sheds new light on how the boundary is established between stem cell daughters and committed leaf primordium cells.

### Chromatin and the Regulation of Cell Proliferation

That polycomb group proteins may be involved in stem cell maintenance has sparked an interest in epigenetic factors that control stem cell behavior through the modification of chromatin (Valk-Lingbeek et al., 2004).

Epigenetic factors that regulate cell proliferation in plant embryos were discussed by Ueli Grossniklaus (University of Zürich). The MEA, FIS, FIE, and MSI1 plant proteins associate in a complex and are homologs of the fly *Polycomb* Repressive Complex 2 (PRC2). The paternal allele of the gene encoding the SET domain protein MEA is inactive after fertilization (Reyes and Grossniklaus, 2003). To understand the nature of this imprinting, the investigators looked for natural variants of the *MEA* gene promoter and found that imprinting was not dependent on epigenetic attractors such as a repeat downstream of *MEA* or on an upstream transposon (Spillane et al., 2004). MEA is able to bind to its own promoter and thus can autoregulate its expression as the amount of transcript increases. Several variants of the PRC2 complex (but no PRC1 components) appear to exist in plants (Reyes and Grossniklaus, 2003). Local manipulation of PRC2 components may shed light on their involvement in plant stem cell biology.

Willy Gruissem (ETH Zürich) described the phenotypes conferred by null alleles of the *RETINOBLASTOMA RELATED* (*RBR*) gene (Ebel et al., 2004). He pointed out similarities to PRC2 complex mutants and demonstrated that the MSI1 protein, which interacts with the retinoblastoma protein, resides in the PRC2 complex (Kohler et al., 2003). MSI1 also forms a complex with the chromatin assembly factor subunits FAS1 and FAS2, which are required for maintenance of expression of patterning genes in shoot and root stem cell organizers (Kaya et al., 2001). Gruissem also described *fas* mutants, which exhibit defects in S phase-related transcription and euchromatin compaction. Together with data on root stem cell sensitivity to RBR activity, these observations reveal connections between chromatin remodeling, the Retinoblastoma protein pathway, and plant stem cell maintenance. The striking possibility is that these factors all contribute to stem cell regulation through epigenetic modifications.

### A View from Animal Stem Cell Research

One of the most attractive aspects of this meeting was the very active participation of animal stem cell biologists. Allan Spradling (Carnegie Insitute, Washington) discussed the lessons that plant biologists can learn from stem cells of the fruit fly. His work on the fly germline stem cell niche reveals that germline stem cells receive signals from the organizing cells that surround them and that intrinsic factors suppress their differentiation (Ohlstein et al., 2004). Signals from organizing cells ensure that the BAM transcription factor family, which promotes differentiation, remains inactive in

germline stem cells. Spradling's microarray analysis of purified fly germline stem cells identified several new candidate regulatory pathways. Polycomb group proteins such as Psc (the fly homolog of Bmi1, which has been implicated in mammalian stem cell maintenance) exhibit differential expression in fly germline stem cells; Psc loss affects stem cell function. These data implicate epigenetic regulation—already well-established for control of mammalian stem cells—as an important mechanism for specifying fly germline stem cells. Spradling contrasted the stability of germline stem cell organizing cells with the more dynamic nature of other organizers, such as those of plant stem cells.

Continuing the epigenetic perspective, Edith Heard (Curie Institute, Paris) reviewed our current understanding of mouse X chromosome inactivation. This process involves coating of one of the female mouse X chromosomes by Xist RNA, histone modification, and Polycomb group protein activity. Early inactivation of the paternal X chromosome is followed by reactivation and subsequent random X inactivation in the early embryo. In mouse embryonic stem cells, Xist chromosome "painting" is followed by histone modifications, some of which are mediated by Polycomb complexes. Heard compared this finding to new in vivo observations on sequential Xist coating, RNA polymerase exclusion, loss of active-chromatin-associated histone marks, onset of Polycomb group gene expression, and acquisition of repressive marks (Okamoto et al., 2004). Different kinetics of X chromosome reactivation during nuclear transfer to oocytes, the notion that X chromosomes remain silent in adult stem cells, and the issue of whether germ cells in the mouse embryo escape X inactivation all illustrate that X inactivation research can provide valuable insights into epigenetic modifications in stem cells.

Austin Smith (University of Edinburgh) questioned the view that tissue culture stem cells and stem cells in vivo are equivalent. He described the nuances of deriving and culturing mouse embryonic stem cells. Smith then discussed evidence that the transcription factors Oct4 and Nanog are required for self-renewal, blocking inappropriate lineage commitment (Ying et al., 2003; Chambers et al., 2003). Nanog and Oct4 overexpression both led to stimulation of symmetric divisions in mouse embryonic stem cells. Smith used homogenous mouse embryonic stem cells in culture to follow their differentiation into neural stem cells. He then compared transcription profiles of these in vitro generated neural stem cells with those for neural stem cells directly isolated from mouse brain tissue. The profiling data imply that the in vitro-generated neural stem cells may not have a counterpart in vivo. Similarly, cultured mouse embryonic stem cells express the oncogenic Ras variant Eras, whereas no role for this variant has been found in stem cells in vivo. Smith urges caution in drawing conclusions about stem cell characteristics from cultured embryonic stem cells.

Taking a regeneration perspective, Alejandro Sánchez Alvarado (University of Utah) discussed the properties of neoblasts, a stem cell-like population in the flatworm *Schmidtea mediterranea*. These flatworms exhibit a remarkable capacity for regeneration and respond to starvation by allometric shrinkage. Both properties suggest that stem cells within the organism respond dramatically to changes in the environment. Neoblasts are characterized by decondensed chromatin and are found throughout the flatworm (except in front of the photoreceptor and in the pharyngeal areas). When regeneration is induced, BrdU labeling stains dividing neoblasts, demonstrating that their daughter cells move to sites of damage to instigate tissue repair. Alvarado's team identified several genes associated with neoblast-mediated regeneration, including a member of the *ARGONAUTE* gene family. ARGONAUTE proteins associate with small RNAs to regulate mRNAs and chromatin and have been implicated in stem cell maintenance in both the model plant *Arabidopsis* and in the fruit fly. Knocking down expression of the *ARGONAUTE* gene using RNA interference (RNAi) revealed a regeneration defect, that is, the neoblasts disappeared and the worms died (Reddien et al., 2005).

## Conclusions

Due to their precise locations, plant stem cells can readily be studied in vivo. The unique patterning pathways that position stem cells in shoots and roots are now being elucidated. In addition, the first hints of the involvement of cell-cycle regulators and chromatin factors in maintenance of the stem cell state in both plants and animals are emerging. Although different regulators seem to pattern stem cell niches in plants and animals, maintenance of the stem cell state in both plants and animals shows commonalities. There are still many issues that need to be explored. Among these are the reasons for different chromatin complexes between plants and animals and the identity of intrinsic factors that regulate plant stem cells. Did such factors escape attention or is the plant stem cell state a quantitative trait? The connections forged between animal and plant biologists at this meeting—the last scientific meeting to be held at the Juan March Foundation in Madrid—demonstrate that we are on the road to answering some of the many exciting questions facing plant and animal stem cell researchers.

### Acknowledgments

The Juan March Foundation meeting entitled "Plant stem cells: independent inventions and conserved mechanisms" was held from 23 to 25 May, 2005, in Madrid, Spain. Co-sponsored by the European Molecular Biology Organization, the meeting was organized by Crisanto Gutierrez, Robert Sablowski and Detlef Weigel.

Over the last 13 years, the Juan March Foundation has hosted 205 scientific conferences on a wide range of topics for scientists from around the world. The meetings are always limited to 50 participants to encourage dialog and discussions that complement the formal presentations. Everyone fortunate enough to have attended one of these meetings enjoyed the interactive and convivial atmosphere, and the scientific enterprise in Spain and worldwide has benefited. The Juan March Foundation has decided to redirect its funding to other projects, although some of the newly developed "Cantoblanco Workshops on Biology" organized by Spanish research centers are still sponsored by the Foundation. Hopefully, other funding agencies will take up the challenge, enabling continuation of these highly interactive and compelling meetings.

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